

**Patent**

Attorney Docket No: AM-00106.P.1-US

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

Angel Cebolla Ramirez et al.

Examiner: To be determined

Group Art Unit: To be determined

Application Number: To be determined

Filed: Herewith

For: **REGULATED EXPRESSION OF  
CLONED GENES USING A CASCADE  
GENETIC CIRCUIT**

Assistant Commissioner for Patents  
Washington D.C., 20231

Sir,

**PRELIMINARY AMENDMENT NO. 1**

Prior to examination, please amend the application as provided below:

**IN THE CLAIMS**

Please cancel all of the claims, claims 1 through 16.

Please add the following claims, claims 17 through 47.

Thus, claims 17 through 47 are pending upon entry of this amendment.

AM-00106.P.1-US

Preliminary Amendment No. 1

- - 17. A cascade genetic circuit, comprising:

- a) a plurality of transcriptional regulators encoded on one or more nucleic acid constructs; and  
wherein expression of an upstream transcriptional regulator from said plurality of transcriptional regulators stimulates expression of at least one downstream transcriptional regulator from said plurality of transcriptional regulators;  
wherein at least two of said plurality of transcriptional regulators are responsive to an inducer;
- b) a target promoter;  
wherein said target promoter is responsive to a downstream transcriptional regulator of said plurality of transcriptional regulators.

- 18. The cascade genetic circuit of claim 17, wherein at least two of said plurality of transcriptional regulators are responsive to the same inducer.
- 19. The cascade genetic circuit of claim 17, wherein said inducer comprises a benzoate derivative.
- 20. The cascade genetic circuit of claim 19, wherein said benzoate derivative is a substituted salicylate molecule or salicylate.
- 21. The cascade genetic circuit of claim 17, wherein at least one of said plurality of transcriptional regulators comprises a polypeptide.
- 22. The cascade genetic circuit of claim 17, wherein at least one of said plurality of transcriptional regulators is encoded by an exogenous nucleic acid molecule.
- 23. The cascade genetic circuit of claim 17, wherein at least two of said plurality of transcriptional regulators are encoded on the same or different exogenous nucleic acid molecules.
- 24. The cascade genetic circuit of claim 17, wherein the expression of at least one of said plurality of transcriptional regulators is modulated by a nucleic acid molecule having the transcription modulating activity of *nahR*.
- 25. The cascade genetic circuit of claim 17, wherein the expression of at least one of said plurality of transcriptional regulators is modulated by a nucleic acid molecule having the transcription modulating activity of *Psal*.

26. The cascade genetic circuit of claim 17, wherein the expression of at least one of said plurality of transcriptional regulators is modulated by a nucleic acid molecule having the transcription modulating activity of *XylS2*.
27. The cascade genetic circuit of claim 17, wherein said target promoter comprises a nucleic acid molecule having the promoter activity of *Pm*.
28. The cascade genetic circuit of claim 17, wherein at least one of said at least two transcriptional regulators regulates the expression of at least one other of said at least two transcription regulators that is responsive to the same inducer.
29. The cascade genetic circuit of claim 17, wherein said inducer modulates at least one of said plurality of transcriptional regulators that regulates the activity of said target promoter.
30. The cascade genetic circuit of claim 17, wherein the activity of said target promoter is multiplicative.
31. The cascade genetic circuit of claim 17, wherein said target promoter regulates the expression of a moiety of interest.
32. The cascade genetic circuit of claim 17, wherein said target promoter is operably linked to a nucleic acid molecule that encodes a moiety of interest.
33. The cascade genetic circuit of claim 32, wherein said nucleic acid molecule that encodes a moiety of interest is exogenous to a genome or endogenous to a genome.
34. A cell comprising a cascade genetic circuit, wherein said cascade genetic circuit comprises:
  - a) a plurality of transcriptional regulators encoded on one or more nucleic acid constructs; and
    - wherein expression of an upstream transcriptional regulator from said plurality of transcriptional regulators stimulates expression of a downstream transcriptional regulator from said plurality of transcriptional regulators;
    - wherein at least two of said plurality of transcriptional regulators are responsive to an inducer;
  - b) a target promoter;
    - wherein said target promoter is responsive to a downstream transcriptional regulator of said plurality of transcriptional regulators.

35. The cell of claim 34, wherein said cell is a prokaryotic cell.
36. The prokaryotic cell of claim 35, wherein said prokaryotic cell is a bacterial cell.
37. The bacterial cell of claim 36, wherein said bacterial cell is a gram-negative bacterial cell.
38. The cell of claim 34, wherein said cell is a eukaryotic cell.
39. The cell of claim 38, wherein said eukaryotic cell is selected from the group consisting of mammalian cells, insect cells, yeast cells and plant cells.
40. A method regulating the expression of a nucleic acid molecule, comprising:
- a) providing or establishing a cascade genetic circuit;
  - b) placing said nucleic acid molecule under control of a target promoter; and
  - c) inducing said cascade genetic circuit to regulate the expression of said nucleic acid molecule.
41. The method of claim 40, wherein said nucleic acid molecule encodes a moiety selected from the group consisting of a hormone, an enzyme, a growth factor, a apolipoprotein, a therapeutic protein, a diagnostic molecule, a diagnostic protein, a diagnostic reporter molecule, a reporter molecule, an anti-sense molecule, a ribozyme, an rRNA, a tRNA, an snRNA, and portions or derivatives thereof.
42. A moiety made by the method of claim 40.
43. A method of making a moiety, comprising:
- a) providing or establishing a cascade genetic circuit;
  - b) placing a nucleic acid molecule that encodes a polypeptide under control of a target promoter; and
  - c) inducing said cascade genetic circuit to regulate the expression of said polypeptide.
44. The method of claim 43, wherein said cascade genetic circuit is provided within a cell.
45. The method of claim 43, wherein said cascade genetic circuit is provided in vitro.

46. The method of claim 43, wherein said nucleic acid molecule encodes a moiety selected from the group consisting of a hormone, an enzyme, a growth factor, a apolipoprotein, a therapeutic protein, a diagnostic molecule, a diagnostic protein, a diagnostic reporter molecule, a reporter molecule, an anti-sense molecule, a ribozyme, an rRNA, a tRNA, an snRNA, and portions or derivatives thereof.
47. A moiety made by the method of claim 43.- -

### REMARKS

#### THE AMENDMENTS TO THE CLAIMS

Applicants have canceled claims 1 through 16 and added claims 17 through 47. These claims add no new subject matter as they are fully supported throughout the specification. For example, claims 17 through 47 are supported by the claims as originally filed

### CONCLUSION

Applicants respectfully submit that the claims are ready for examination and in condition for allowance. Please apply any charges not covered, or any credits to **Deposit Account 501321 in the name of David R. Preston & Associates having Customer No.: 24232.**

Respectfully submitted,

Date:

12/21/00



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